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(54) **Method for selectively increasing the ratio of single major components of antibiotic A 40926 complex.**

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(73) Proprietor: **GRUPPO LEPETIT S.p.A.**
Via G. Murat 23
I-20159 Milano(IT)

(72) Inventor: **Selva, Enrico**
15, Via Di Vittorio
I-27027 Gropello Cairoli (PV)(IT)
Inventor: **Denaro, Maurizio**
41, Viale Bligny
I-20136 Milano(IT)
Inventor: **Gastaldo, Luciano**
7, Via San Martino
I-20010 Pogliano Milanese (MI)(IT)
Inventor: **Cassani, Giovanni**
3, Via Vittadini
I-27100 Pavia(IT)
Inventor: **Parenti, Francesco**
24, Via Benvenuto Cellini
I-20020 Lainate (MI)(IT)

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Description

Antibiotic A 40926 is a glycopeptidic antibiotic which has been isolated from a culture of Actinomadura, named Actinomadura sp. ATCC 39727. It is a complex whose factors have been named factor A, factor B, factor B_0, factor PA and factor PB. It was described in EP-A-177882.

This antibiotic complex as well as the single factors thereof bind to D-Alanyl-D-Alanine terminating oligopeptides and are mainly active against gram-positive bacteria and Neisseriae.

The present invention is directed to a method for selectively enhancing the production of factors A, and/or B_0 of antibiotic A 40926 either to isolate these single components in better yields or to enrich the complex in one or both the above components, which comprises adding an appropriate precursor of the desired antibiotic factor to an A 40926 producing culture during fermentation.

According to the method of the invention, it is in fact possible, for instance, to modulate the ratio of the single major components of antibiotic A 40926 complex in large scale industrial fermentation. This method therefore represents a useful tool to adjust the composition of the final product to adhere to standard specifications.

Moreover, by following the procedure of the invention it is also possible to obtain, directly from the fermentation mass of the producing strain, a crude product very rich in antibiotic A 40926 factor A or B_0 which can then be isolated in a pure form with higher yields and less time consuming steps.

A further object of the present invention is a method for enhancing the production of A 40926 factors PA and/or PB. It is known from EP-A- 177882 that these two factors are natural "precursors" of antibiotic A 40926 factors A and B_0, respectively. Therefore, by conducting the recovery of the antibiotic substances with a limited exposition to basic conditions, a complex will be obtained which is enriched in factors PA and/or PB instead of factor A and/or B_0. More particularly, the appropriate precursor enhancing the production of antibiotic A 40926 factors PA is the same as that for antibiotic A 40926 factor A and the appropriate precursor for factor PB is the same as that for antibiotic A 40926 factor B_0.

The appropriate precursor for increasing the ratio of factor B_0 in antibiotic A 40926 complex is selected from valine, its salts with acids and bases which are non-toxic to the producing microorganism, alpha-keto-isovaleric acid, its salts with bases which are non-toxic to the producing microorganism, its esters with mono-, and poly-hydroxy lower alkanols, isobutyric acid, its salts with bases which are non-toxic to the producing microorganism, its esters with mono- and poly-hydroxy lower alkanols, isobutanol and its esters with acids which are non-toxic to the producing microorganism.

The appropriate precursor for increasing the ratio of factor A in antibiotic A 40926 complex is selected from n-propanol and its esters which are non-toxic to the producing microorganism, propionic acid, its salts with bases which are non-toxic to the producing microorganism, its esters with mono- or poly-hydroxy lower alkanols, isoleucine, its salts with acids and bases which are non-toxic to the producing microorganism, alpha-keto-beta-methylvaleric acid, its salts with bases which are non-toxic to the producing microorganism, its esters with mono- and poly-hydroxy lower alkanols, 2-methylbutyric acid, its salts with bases which are non-toxic to the producing microorganism, its esters with mono- and poly-hydroxy lower alkanols, 2-methylbutanol and its esters with acids which are non-toxic to the producing microorganism, and any-other substance which is capable of being transformed into propionyl-coenzyme A under the fermentation conditions.

Salts with bases which are non-toxic to the microorganism are salts wherein the type and concentration of the given cation is such that it does not impair either the growth of the microorganism culture or the production of the desired antibiotic substance to a considerable extent at the concentration employed in the fermentation mass. Examples of said cations are those from alkali metals and alkaline earth metals such as sodium, potassium, calcium or magnesium, as well as those from amines, such as ammonium, primary, secondary or tertiary (C_1-C_4)alkyl ammonium and hydroxy(C_1-C_4)alkyl ammonium. Preferred salts are those with sodium, potassium or ammonium ions.

Examples of salts with acids which are non-toxic to the producing microorganism, i.e. salts with acids which do not either impair considerably the growth of the microorganism culture or the production of the desired antibiotic substance, at the concentration at which they are present in the fermentation mass, are preferably mineral acids such as hydrochloric acid, even if also organic acids may, in some instances, be present.

Esters of an appropriate precursor as defined above with mono- and poly-hydroxy lower alkanols ar esters with (C_1-C_6)alkanols with 1, 2, 3, 4, 5 or 6 hydroxy functions per molecule. When (C_1-C_4)alkanols are used, they must be different from those which act as precursors for the other antibiotic factor (i.e. isobutanol or 2-methylbutanol) unless concomitant increase of both factors is desired.

Preferred examples of poly-hydroxy alkanols are glycerol and propylene glycol.

When the lower alkanol may be present in different enantiomeric and epimeric forms, in the present description and claims, each single form separately as well as the mixture of said single forms in any proportion is intended.

Esters of an appropriate hydroxy containing precursor as defined above which are non-toxic to the microorganism are (C_2-C_{22})alkanoyl esters wherein the type and concentration of the alkanoyl moiety in the fermentation medium is such that it does not impair the growth of the microorganism culture or the production of the desired antibiotic substance to a considerable extent. In general, straight chain (C_2-C_4)-alkanols are preferred.

An antibiotic A 40926 producing culture is a culture of a strain like Actinomadura sp. ATCC 39727 or a producing mutant or variant thereof, which is capable, upon cultivation, of producing recoverable amounts of antibiotic A 40926.

The method of the invention includes cultivating an antibiotic A 40926 producing culture in an aqueous nutrient culture medium containing an assimilable source of carbon, an assimilable source of nitrogen and inorganic salts under the usual conditions known for the cultivation of Streptomycetales in general and for the A 40926 producing strains in particular (c.f. also EP-A-177882 cited above) and adding an effective amount of the appropriate precursor to selectively enhance the production of antibiotic A 40926 factor A and/or factor B_0.

The appropriate precursor may be added to the fermentation in a continuous or discontinuous way during fermentation, or in pre-culture, or may be added to the cultivation medium before fermentation. It may be added directly, if suitably fluid at the fermentation temperature, or it may be added as a solution, suspension or emulsion, and preferably it is an aqueous solution or suspension.

An "effective amount" of appropriate precursor means an amount of precursor as defined above which, when added to the fermentation, gives a concentration of a selective precursor sufficient to produce the selective increase of the specific factor of antibiotic A 40926, without causing toxic effects to the growing culture of the producing microorganism.

The rate of addition of the precursor must be high enough to increase the yield of the desired factor to a considerable or optimum extent without however producing a toxic effect on the fermentation.

In general, it may be useful to feed an effective amount of the appropriate precursor in a continuous way or portionwise at the beginning, or during the production stage of the fermentation.

In some instances, it may be convenient to feed a mixture of some or all of the precursors of a certain factor in order to obtain a maximum result with minimum "toxic" effects on the culture.

Following fermentation, if desired, antibiotic A 40926 complex or the single factors A or B_0, PA or PB can be recovered according to the known procedures or obvious modifications thereof.

The nutrient fermentation media suitable for the fermentation of the A 40926 producing strain which can be used in the method of the invention, usually contain: a suitable carbon source which, for instance, may be selected from sugars (e.g. glucose, sucrose, maltose), polysaccharides (e.g. starch, dextrane) polyalcohols (e.g. glycerol, propylene glycol); suitable nitrogen sources which, for instance, may be selected from ammonium salts, asparagine, peanut meal, soybean meal, meat extract, tryptone, peptone, yeast hydrolyzate, yeast extract and corn steep liquor; and inorganic salts. Among the inorganic salts which can be incorporated in the culture media there are the customary soluble salts capable of yielding sodium, potassium, iron, zinc, cobalt, magnesium, calcium, ammonium, chloride, carbonate, sulfate, phosphate, nitrate and the like ions.

Ordinarily, the antibiotic-producing strain is pre-cultured in a shake flask, then the culture is used to inoculate jar fermentors for production of substantial quantities of the antibiotic substances. The medium used for the pre-culture can be the same as that employed for larger fermentations, but other media can also be employed.

The fermentation is carried out for a time varying from 50 to 150 hours under submerged aerobic conditions at a temperature between 25 °C and 35 °C, preferably between 27 °C and 33 °C. The addition of the selectively effective amount of appropriate precursors can be made to the fermentative media before inoculation of the producing strain, or 24 to 48 hours after the fermentation is started. The addition may be made in one or several portions or in a continuous way.

According to a typical experiment embodying this invention, a culture of the A 40926 producing strain, maintained on oat-meal agar slants, is inoculated into a flask containing 100 ml of a vegetative medium. After about 72 hours, samples of the culture (5 millilitres) are used to inoculate a series of fermentation flasks containing 100 ml of fermentative medium , to which a selectively effective amount of precursor is added as appropriate. If concomitant increase of the two factors of A 40926 complex is desired, the appropriate precursors are added to the same fermentation flask. The fermentation is continued for additional 60 to 150 hours, and it is monitored at intervals by HPLC, then the fermentation cake is removed

and samples of the broth are analyzed by HPLC.

The recovery of the antibiotic substances may be carried out as known in the art and described in detail in EP-A- 177882.

For veterinary application, the whole fermentation cake or concentrated broth can be used.

The addition of the precursor to the fermentation is such that it does not affect considerably its predetermined pH range. Thus, for instance, when free acid precursors are added directly to the medium, the pH is maintained under control by buffering the medium or by immediate neutralization with bases which are non-toxic to the microorganism.

When the precursor to be added is an aminoacid, it may be supplied to the fermentation as an aqueous solution of its salts with acids or bases which are non-toxic to the producing microorganism, e.g. hydrochlorides and sodium salts, even if in many instances the aminoacid may conveniently be added as a solution of the "internal salt". Both racemic mixtures and optically active isomers can be used as precursors.

However, in general, the addition of the L-form gives higher yields than the corresponding D-form.

A preferred embodiment of the process of this invention is therefore represented by the use of the L-form of the aminoacid precursor for enhancing the concentration of factor B_0 or PB (L-valine, a salt or an ester thereof), and/or factor A or PA (L-isoleucine, a salt or an ester thereof) of antibiotic A 40926 complex. According this preferred embodiment, it is also possible to increase the percentage of factor A or B_0 in the fermentation product over 80% of the complex.

With lower alkanoic acid precursors (2-methylbutyric acid, isobutyric acid, alpha-keto-isovaleric acid, and alpha-keto-beta-methylvaleric acid) the addition may be made through an aqueous solution of their salts with non-toxic bases; ammonium and sodium salts are usually preferred.

When esters of the above lower alkanoic acids and unsaturated fatty acids with mono-hydroxy lower alkanols are employed as precursors, said esters are usually derived from methanol, ethanol and propanol, although esters with C_4-C_6 alkanols may also be employed. In this case, the C_4-C_6 alkanol must be different from that which may act as precursor for the other factor, (isobutanol, 2-methylbutanol or propanol, unless concomitant increase of the other factor is desired.

Alkanol precursors such as isobutanol, 2-methylbutanol and n-propanol are usually added as such to the fermentation. However, they can be supplied also as esters of acids which are non-toxic to the microorganism. These acids must be different from those which may act as precursors for the other A 40926 factor unless concomitant increase of the other factor is desired. Usually, esters with linear (C_2-C_4) alkanoic acids such as acetic, propionic and butyric acid are preferred.

The "selectively effective amount" to be added to the fermentation medium according to this invention depends on the type of precursor. Usually, with the esters of the lower alkanoic acids (isobutyric acid, 2-methylbutyric acid) amounts that yield a concentration of the acid into the fermentation medium ranging between 0.1 g/l and 5 g/l are employed, with the range between 0.1 g/l and 1 g/l being preferred. With lower alkanols (isobutanol, 2-methylbutanol, n-propanol) or their esters with acids which are non-toxic to the microorganism, amounts that yield a concentration of the alcohol ranging between 0.5 g/l and 5 g/l are usually employed, with the range between 1 g/l and 2 g/l being preferred.

With the aminoacids (valine, isoleucine) and the keto-acids (alpha-keto-isovaleric acid, alpha-keto-beta-methylvaleric acid) or their salts with acids and bases the "selectively effective amount" added to the fermentation medium usually ranges between 0.2 g/l and 5 g/l, and preferably between 0.5 g/l and 4 g/l; the most preferred range being between 2 and 4 g/l.

In the case where the lower alkanoic acids (e.g. isobutyric acid, 2-methylbutyric acid), or their salts are directly added to the fermentation medium, the "selectively effective amount" usually ranges between 0.1 g/l and 2.5 g/l, with the range between 0.3 g/l and 1.5 g/l being preferred.

Concentrations higher than those indicated above may still be effective in enhancing the relative percentage of one of the A 40926 factors but, in general, the overall yield is depressed because of toxic effects on the culture.

Example 1

A culture of Actinomadura sp. ATCC 39727 on agar slant is used to inoculate a 500 ml Erlenmeyer flask containing 100 ml of the following m dium:

Meat extract	5 g
Autolysed yeast	5 g
Peptone	5 g
Casein hydrolysed	3 g
Glucose	20 g
NaCl	1.5 g
$CaCO_3$	4 g
Distilled water q.s.	1000 ml

The flask is incubated at 28° C on a rotary shaker at 200 rpm for about 72 hours and then the mycelium is collected by centrifugation, washed twice with sterile deionized water and suspended in 100 ml of sterile deionized water. 2 ml of this suspension are used to inoculate a 500 ml Erlenmeyer flask containing 100 ml of the above medium and the appropriate precursor is added. The antibiotic production is monitored by the paper-disc agar diffusion method using B. subtilis on a minimal medium as the test organism.

After 72 hours of cultivation on a rotary shaker at 200 rpm, the fermentation cake is removed by filtration and the filtrate is passed through a Sepharose-Epsilon-aminocaproyl-D-Alanyl-D-Alanine column (0.5 ml of resin/0.3 ml of broth) and eluted with 1% (w/v) ammonia hydrate. The fractions which contain antibiotic A 40926 are pooled and left one day at room temperature, then are analysed by HPLC according to the following procedure:

column: Silanized silica gel Ultrasphere ODS (5 micrometer) Altex (Beckman) 4.6 mm (i.d.) x 250 mm

pre-column: Silanized silica gel Brownlee Labs RP 18 (5 micrometer)

eluent A: CH_3CN 10% } adjusted at
(2.5 g/l) $NaH_2PO_4.H_2O$ 90% } pH 6.0

eluent B: CH_3CN 70% } adjusted at
(2.5 g/l) $NaH_2PO_4.H_2O$ 30% } pH 6.0

elution: linear gradient from 5% to 60% of eluent B in eluent A, in 40 min

flow rate: 1.8 ml/min

U.V detector: 254 nm

internal standard: Teicoplanin A_2 component 2, R_t = 20.3 min (Gruppo Lepetit S.p.A.)

relative retention times:
A 40926 factor A 1.12
A 40926 factor B_0 1.22
A 40926 factor B_1 1.27
A 40926 factor PA 1.15
A 40926 factor PB 1.27

Percentage distribution

The components are separated by the above procedure and their relative distribution is obtained as a percent of the total of the two peaks by the area percentage method. The results of representative experiments are reported below:

Precursor added (nM)	Total conc. (microgram/ml)	Factor A % Factor A+Factor B_0	Factor B_0 % Factor A+Factor B_0
None (-)	24-60	22-36	78-64
L-valine 8	24	5	95
L-isoleucine 8	13	59	41
Isobutanol 5	30-57	3-18	97-81
n-Propanol 5	34	51	48
2-Methyl-1-butanol 5	34	47	53

By essentially following the above procedure but rapidly neutralizing the ammonic luates instead of leaving them aside for 24 h, the antibiotic A 40926 factors PA and/or PB are obtained, instead of factors A and B_0, respectively.

The percentages and the results are substantially as reported above for factors A and B_0.

Claims

1. A process for preparing antibiotic A 40926 complex enriched in its factors A and/or B_0, or PA and/or PB, which comprises adding to a culture of Actinomadura sp. ATCC 39727 or an A 40926 producing mutant thereof a selectively effective amount of the appropriate precursor, wherein the appropriate precursor for increasing the ratio of factor A or PA in antibiotic A 40926 complex is selected from n-propanol and its esters which are non-toxic to the producing microorganism, propionic acid, its salts with bases which are non-toxic to the producing microorganism, its esters with mono- or poly-hydroxy lower alkanols, isoleucine, its salts with acids and bases which are non-toxic to the producing microorganism, alpha-keto-beta-methylvaleric acid, its salts with bases which are non-toxic to the producing microorganism, its esters with mono- and poly-hydroxy lower alkanols, 2-methylbutyric acid, its salts with bases which are non-toxic to the producing microorganism, its esters with mono- and poly-hydroxy lower alkanols, 2-methylbutanol and its esters with acids which are non-toxic to the producing microorganism, and any other substance which is capable of being transformed into propionyl-coenzyme A under the fermentation conditions, and the appropriate precursor for increasing the ratio of factor B_0 or PB in antibiotic A 40926 complex is selected from valine, its salts with acids and bases which are non-toxic to the producing microorganism, alpha-keto-isovaleric acid, its salts with bases which are non-toxic to the producing microorganism, its esters with mono- and poly-hydroxy lower alkanols, isobutyric acid, its salts with bases which are non-toxic to the producing microorganism, its esters with mono- and poly-hydroxy lower alkanols, isobutanol and its esters with acids which are non-toxic to the producing microorganism.

2. A process is claimed in claim 1 wherein the appropriate precursor added is valine or its salts with acids and bases non-toxic to the microorganism and the respective selectively effective amount is between 0.2 g/l and 5 g/l, preferably between 0.5 g/l and 4 g/l.

3. A process as claimed in claim 1 wherein the appropriate precursor added is isobutyric acid or its salts with bases non-toxic to the microorganism and the respective selectively effective amount is between 0.1 g/l and 2.5 g/l, preferably between 0.3 g/l and 1.5 g/l.

4. A process as claimed in claim 1 wherein the appropriate precursor added is an ester of isobutyric acid with a mono- or poly-hydroxy lower alkanol and the respective selectively effective amount is between 0.1 g/l and 5 g/l, preferably between 0.1 g/l and 1 g/l.

5. A process as claimed in claim 1 wherein the appropriate precursor added is isobutanol or its esters with acids non-toxic to the microorganism and the respective selectively effective amount is between 0.5 g/l and 5 g/l, preferably between 1 g/l and 2 g/l.

6. A process as claimed in claim 1 wherein the appropriate precursor added is isoleucine or its salts with acids and bases non-toxic to the microorganism and the respective selectively effective amount is between 0.2 g/l and 5 g/l, preferably between 0.5 g/l and 4 g/l.

7. A process as claimed in claim 1 wherein the appropriate precursor added is 2-methylbutyric acid or its salts with bases non-toxic to the microorganism and the respective selectively effective amount is between 0.1 g/l and 2.5 g/l, preferably between 0.3 g/l and 1.5 g/l.

8. A process as claimed in claim 1 wherein the appropriate precursor added is an ester of 2-methylbutyric acid with a mono- or poly-hydroxy lower alkanol and the respective selectively effective amount is between 0.1 g/l and 5 g/l, preferably between 0.1 g/l and 1 g/l.

9. A process as claimed in claim 1 wherein the appropriate precursor added is 2-methylbutanol, n-propanol, or their esters with an acid non-toxic to the microorganism and the respective selectively effective amount is between 0.5 g/l and 5 g/l, preferably between 1 g/l and 2 g/l.

10. A process as claimed in claim 1 wherein the appropriate precursor added is alpha-keto-beta-methylvaleric acid, its salts with bases non-toxic to the microorganism or its esters with mono-or poly-hydroxy lower alkanols and the respective selectively effective amount is between 0.2 g/l and 5 g/l, preferably between 2 g/l and 4 g/l.

11. A process as claimed in claim 1, 2, 3, 6, 7, or 10 wherein the salts with bases non-toxic to the microorganism are sodium or ammonium salts.

12. A process as claimed in claim 1, 4, 8 or 10 wherein the ester is an ester with one of the following alkanols: methanol, ethanol, propanol, ethylene glycol and glycerol.

13. A process as claimed in claim 1, 2 or 6 wherein the aminoacid is in the L- form.

14. A process as claimed in claim 1, 2, or 6 wherein the salt with an acid non-toxic to the microorganism is the hydrochloride or the sulfate.

15. A process as claimed in any one of the claims 1, 5 or 9 wherein the ester with an acid non-toxic to the microorganism is an ester with one of the following acids: acetic acid, propionic acid and butyric acid.

16. A process as claimed in any one of the preceding claims wherein the strain is Actinomadura sp. ATCC 39727.

17. A process as claimed in any one of the preceding claims wherein the fermentation is carried out at a temperature between 25 °C and 35 °C, and preferably between 27 °C and 33 °C.

18. A process as claimed in any one of preceding claims wherein the addition of the appropriate precursor is carried out in the pre-culture, at the beginning of the fermentation or 24 to 48 hours after the fermentation is started.

Patentansprüche

1. Verfahren zur Herstellung des antibiotischen Komplexes A 40926, angereichert in seinen Faktoren A und/oder B_0, oder PA und/oder PB, umfassend die Zugabe einer selektiv wirksamen Menge einer geeigneten Vorstufe zu einer Kultur von Actinomadura sp. ATCC 39727 oder einer A 40926 erzeugenden Mutante davon, wobei die geeignete Vorstufe zur Erhöhung des Verhältnisses von Faktor A oder PA im antibiotischen Komplex A40926 ausgewählt ist aus n-Propanol und dessen Estern, die für den erzeugenden Mikroorganismus ungiftig sind, Propionsäure, deren Salzen mit Basen, die für den erzeugenden Mikroorganismus ungiftig sind, deren Estern mit Mono- oder Polyhydroxyniederalkanolen, Isoleucin, dessen Salzen mit Säuren und Basen, die für den erzeugenden Mikroorganismus ungiftig sind, α-Keto-β-methylvaleriansäure, deren Salzen mit Basen, die für den erzeugenden Mikroorganismus ungiftig sind, deren Estern mit Mono- und Polyhydroxyniederalkanolen, 2-Methylbuttersäure, deren Salzen mit Basen, die für den erzeugenden Mikroorganismus ungiftig sind, deren Estern mit Mono- und Polyhydroxyniederalkanolen, 2-Methylbutanol und dessen Estern mit Säuren, die für den erzeugenden Mikroorganismus ungiftig sind, und jedem anderen Stoff, der befähigt ist, unter Fermentationsbedingungen in Propionyl-Coenzym A umgesetzt zu werden, und die geeignete Vorstufe zur Erhöhung des Verhältnisses von Faktor B_0 oder PB im antibiotischen Komplex A 40926 ausgewählt ist aus Valin, dessen Salzen mit Säuren und Basen, die für den erzeugenden Mikroorganismus ungiftig sind, α-Ketoisovaleriansäure, deren Salzen mit Basen, die für den erzeugenden Mikroorganismus ungiftig sind, deren Estern mit Mono- und Polyhydroxyniederalkanolen, Isobuttersäure, deren Salzen mit Basen, die für den erzeugenden Mikroorganismus ungiftig sind, deren Estern mit Mono- und Polyhydroxyniederalkanolen, Isobutanol und dessen Estern mit Säuren, die für den erzeugenden Mikroorganismus ungiftig sind.

2. Verfahren nach Anspruch 1, wobei die zugegebene geeignete Vorstufe Valin ist oder dessen Salze mit Säuren und Basen, die für den Mikroorganismus ungiftig sind, und die entsprechend selektiv wirksame Menge zwischen 0,2 g/l und 5 g/l, vorzugsweise zwischen 0,5 g/l und 4 g/l beträgt.

3. Verfahren nach Anspruch 1, wobei die zugegebene geeignete Vorstufe Isobuttersäure ist oder deren Salze mit Basen, die für den Mikroorganismus ungiftig sind, und die entsprechend selektiv wirksame Menge zwischen 0,1 g/l und 2,5 g/l, vorzugsweise zwischen 0,3 g/l und 1,5 g/l beträgt.

4. Verfahren nach Anspruch 1, wobei die zugegebene geeignete Vorstufe ein Ester von Isobuttersäure mit einem Mono- oder Polyhydroxyniederalkanol ist und die entsprechend selektiv wirksame Menge

zwischen 0,1 g/l und 5 g/l, vorzugsweise zwischen 0,1 g/l und 1 g/l beträgt.

5. Verfahren nach Anspruch 1, wobei die zugegebene geeignete Vorstufe Isobutanol ist oder dessen Ester mit Säuren, die für den Mikroorganismus ungiftig sind, und die entsprechend selektiv wirksame Menge zwischen 0,5 g/l und 5 g/l, vorzugsweise zwischen 1 g/l und 2 g/l beträgt.

6. Verfahren nach Anspruch 1, wobei die zugegebene geeignete Vorstufe Isoleucin ist oder dessen Salze mit Säuren und Basen, die für den Mikroorganismus ungiftig sind, und die entsprechend selektiv wirksame Menge zwischen 0,2 g/l und 5 g/l und vorzugsweise zwischen 0,5 g/l und 4 g/l beträgt.

7. Verfahren nach Anspruch 1, wobei die zugegebene geeignete Vorstufe 2-Methylbuttersäure ist oder deren Salze mit Basen, die für den Mikroorganismus ungiftig sind, und die entsprechend selektiv wirksame Menge zwischen 0,1 g/l und 2,5 g/l, vorzugsweise zwischen 0,3 g/l und 1,5 g/l beträgt.

8. Verfahren nach Anspruch 1, wobei die geeignete Vorstufe ein Ester von 2-Methylbuttersäure mit einem Mono- oder polyhydroxyniederalkanol ist und die entsprechend selektiv wirksame Menge zwischen 0,1 g/l und 5 g/l, vorzugsweise zwischen 0,1 g/1 und 1 g/l beträgt.

9. Verfahren nach Anspruch 1, wobei die zugegebene geeignete Vorstufe 2-Methylbutanol, n-Propanol oder deren Ester mit einer Säure ist, die für den Mikroorganismus ungiftig sind, und die entsprechend selektiv wirksame Menge zwischen 0,5 g/l und 5 g/l, vorzugsweise zwischen 1 g/l und 2 g/l beträgt.

10. Verfahren nach Anspruch 1, wobei die zugegebene geeignete Vorstufe α-Keto-β-methylvaleriansäure ist, deren Salze mit Basen, die für den Mikroorganismus ungiftig sind, oder deren Ester mit Mono- oder Polyhydroxyniederalkanolen und die entsprechend selektiv wirksame Menge zwischen 0,2 g/l und 5 g/l, vorzugsweise zwischen 2 g/l und 4 g/l beträgt.

11. Verfahren nach Anspruch 1, 2, 3, 6, 7 oder 10, wobei die Salze mit Basen, die für den Mikroorganismus ungiftig sind, Natrium- oder Ammoniumsalze sind.

12. Verfahren nach Anspruch 1, 4, 8 oder 10, wobei der Ester ein Ester der nachstehenden Alkanole ist: Methanol, Ethanol, Propanol, Ethylenglykol und Glycerin.

13. Verfahren nach Anspruch 1, 2 oder 6, wobei die Aminosäure in der L-Form vorliegt.

14. Verfahren nach Anspruch 1, 2 oder 6, wobei das Salz mit einer Säure, das für die Mikroorganismen ungiftig ist, das Hydrochlorid oder das Sulfat ist.

15. Verfahren nach einem der Ansprüche 1, 5 oder 9, wobei der Ester mit einer Säure, der für den Mikroorganismus ungiftig ist, ein Ester der nachstehenden Säuren ist: Essigsäure, Propionsäure und Buttersäure.

16. Verfahren nach einem der vorangehenden Ansprüche, wobei der Stamm Actinomadura sp. ATCC 39727 ist.

17. Verfahren nach einem der vorangehenden Ansprüche, wobei die Fermentation bei einer Temperatur zwischen 25 und 35 °C und vorzugsweise zwischen 27 und 33 °C durchgeführt wird.

18. Verfahren nach einem der vorangehenden Ansprüche, wobei die Zugabe der geeigneten Vorstufe während der Vorzüchtung am Beginn der Fermentation oder 24 bis 48 Stunden nach dem Beginn der Fermentation durchgeführt wird.

Revendications

1. Procédé de préparation du compl xe d'antibiotique A 40926 enrichi en ses facteurs A t/ou B_0, ou PA et/ou PB, qui consiste à ajouter, à une culture d'Actinomadura sp. ATCC 39727 ou d'un de ses mutants produisant de l'A 40926, une quantité sélectivement efficace du précurseur approprié, dans lequel le précurseur approprié pour augmenter le rapport du facteur A ou PA dans le complexe d'antibiotique A

40926 est choisi parmi le n-propanol et ses esters qui sont non toxiques pour le micro-organisme producteur, l'acide propionique, ses sels avec des bases qui sont non toxiques pour le micro-organisme producteur, ses esters avec des alcanols inférieurs mono- ou poly-hydroxylés, l'isoleucine, ses sels avec des acides et des bases qui sont non toxiques pour le micro-organisme producteur, l'acide α-céto-β-méthylvalérique, ses sels avec des bases qui sont non toxiques pour le micro-organisme producteur, ses esters avec des alcanols inférieurs mono- et poly-hydroxylés, l'acide 2-méthylbutyrique, ses sels avec des bases qui sont non toxiques pour le micro-organisme producteur, ses esters avec des alcanols inférieurs mono- et poly-hydroxylés, le 2-méthylbutanol et ses esters avec des acides qui sont non toxiques pour le micro-organisme producteur, et n'importe quelle autre substance qui est susceptible d'être transformée en propionyl-coenzyme A dans les conditions de fermentation, et le précurseur approprié pour augmenter le rapport du facteur B_0 ou PB dans le complexe d'antibiotique A 40926 est choisi parmi la valine, ses sels avec des acides et des bases qui sont non toxiques pour le micro-organisme producteur, l'acide α-céto-isovalérique, ses sels avec des bases qui sont non toxiques pour le micro-organisme producteur, ses esters avec des alcanols inférieurs mono- et poly-hydroxylés, l'acide isobutyrique, ses sels avec des bases qui sont non toxiques pour le micro-organisme producteur, ses esters avec des alcanols inférieurs mono- et poly-hydroxylés, l'isobutanol et ses esters avec des acides qui sont non toxiques pour le micro-organisme producteur.

2. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est la valine ou ses sels avec des acides et des bases non toxiques pour le micro-organisme et la quantité sélectivement efficace respective est comprise entre 0,2 g/l et 5 g/l, de préférence entre 0,5 g/l et 4 g/l.

3. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est l'acide isobutyrique ou ses sels avec des bases non toxiques pour le micro-organisme et la quantité sélectivement efficace respective est comprise entre 0,1 g/l et 2,5 g/l, de préférence entre 0,3 g/l et 1,5 g/l.

4. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est un ester de l'acide isobutyrique avec un alcanol inférieur mono- ou poly-hydroxylé et la quantité sélectivement efficace respective est comprise entre 0,1 g/l et 5 g/l, de préférence entre 0,1 g/l et 1 g/l.

5. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est l'isobutanol ou ses esters avec des acides non toxiques pour le micro-organisme et la quantité sélectivement efficace respective est comprise entre 0,5 g/l et 5 g/l, de préférence entre 1 g/l et 2 g/l.

6. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est l'isoleucine ou ses sels avec des acides et des bases non toxiques pour le micro-organisme et la quantité sélectivement efficace respective est comprise entre 0,2 g/l et 5 g/l, de préférence entre 0,5 g/l et 4 g/l.

7. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est l'acide 2-méthylbutyrique ou ses sels avec des bases non toxiques pour le micro-organisme et la quantité sélectivement efficace respective est comprise entre 0,1 g/l et 2,5 g/l, de préférence entre 0,3 g/l et 1,5 g/1.

8. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est un ester de l'acide 2-méthylbutyrique avec un alcanol inférieur mono- ou poly-hydroxylé et la quantité sélectivement efficace respective est comprise entre 0,1 g/l et 5 g/l, de préférence entre 0,1 g/l et 1 g/l.

9. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est le 2-méthylbutanol, le n-propanol ou leurs esters avec un acide non toxique pour le micro-organisme et la quantité sélectivement efficace respective est comprise entre 0,5 g/l et 5 g/l, de préférence entre 1 g/l et 2 g/l.

10. Procédé selon la revendication 1, dans lequel le précurseur approprié ajouté est l'acide α-céto-β-méthylvalérique, ses sels avec des bases non toxiques pour le micro-organisme ou ses esters avec des alcanols inférieurs mono- ou poly-hydroxylés et la quantité sélectivement efficace respective est comprise entre 0,2 g/l et 5 g/l, de préférence entre 2 g/l et 4 g/l.

11. Procédé selon la revendication 1, 2, 3, 6, 7 ou 10, dans lequel les sels avec des bases non toxiques pour le micro-organisme sont les sels de sodium ou d'ammonium.

12. Procédé selon la revendication 1, 4, 8 ou 10, dans lequel l'ester est un ster avec un des alcanols suivants : méthanol, éthanol, propanol, éthylène glycol et glycérol.

13. Procédé selon la revendication 1, 2 ou 6, dans lequel l'amino-acide est sous la forme L.

14. Procédé selon la revendication 1, 2 ou 6, dans lequel le sel avec un acide non toxique pour le micro-organisme est le chlorhydrate ou le sulfate.

15. Procédé selon l'une quelconque des revendications 1, 5 et 9, dans lequel l'ester avec un acide non toxique pour le micro-organisme est un ester avec un des acides suivants : acide acétique, acide propionique et acide butyrique.

16. Procédé selon l'une quelconque des revendications précédentes, dans lequel la souche est Actinomadura sp. ATCC 39727.

17. Procédé selon l'une quelconque des revendications précédentes, dans lequel la fermentation est effectuée à une température comprise entre 25 °C et 35 °C, et de préférence entre 27 °C et 33 °C.

18. Procédé selon l'une quelconque des revendications précédentes, dans lequel l'addition du précurseur approprié est effectuée dans la pré-culture, au début de la fermentation ou 24 à 48 heures après le démarrage de la fermentation.